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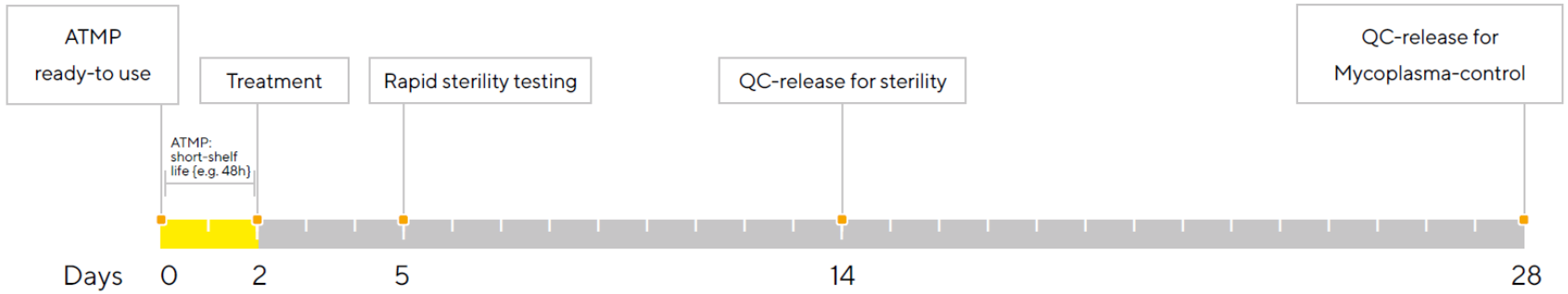
Simplifying Progress

Microsart® ATMP Extraction
Microsart® ATMP Bacteria & Fungi

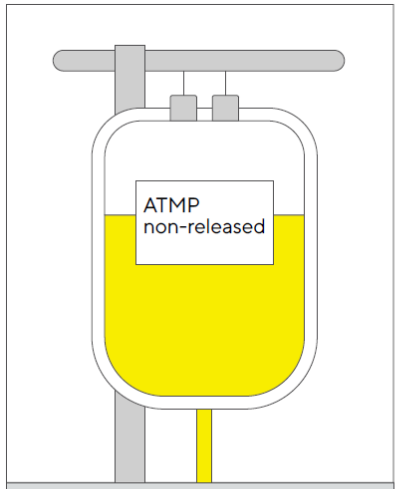
Microsart® ATMP Sterile Release

2022

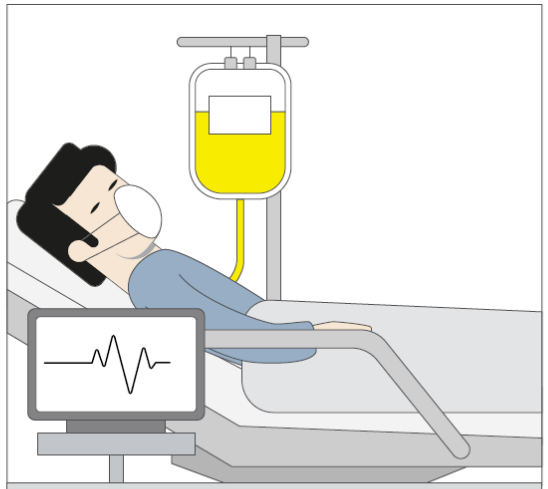
ATMPs put microbiological QC to novel challenges



5, 14 or 28 days
of waiting
is too long
for ATMPs!



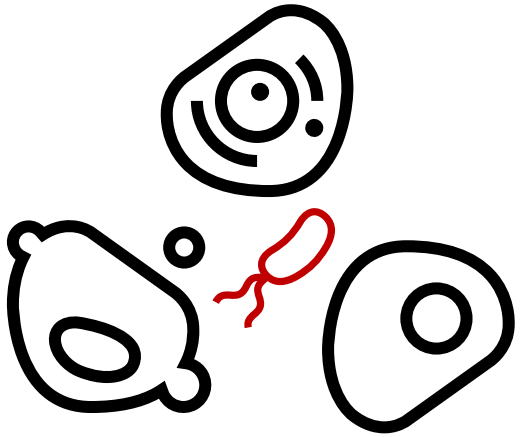
QC result pending



Treatment despite risk:
ATMP might be contaminated

Patient's life at risk

Nucleic acid techniques

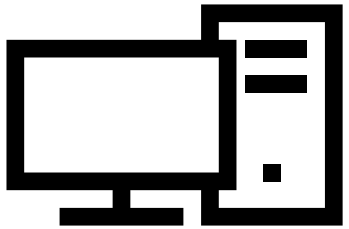


ATMP
(CHO, HEK,...)

DNA extraction
of a small sample

DNA

Amplification
of target sequence



Detection in
real-time PCR cycler:
Contamination? Yes/No

Results within 3 hours!

Bacteria & fungi contamination detection

Microsart® ATMP Extraction

- **Real-time PCR allows detection of bacteria and fungi**
  - In 3 h
  - Down to 2.5-99 CFU/ml
- **Validated combination**
  - In accordance with EP 5.1.6, USP 1223, EP 2.6.27, and USP 1071
- **Support**
  - ***Product Validation Report*** containing all experimental details
  - ***Matrix Validation Proposal*** giving an overview of the required set up and materials
  - ***Matrix Validation Template*** containing detailed information for the customer specific matrix validation
  - **Technical support** during matrix validation process

Microsart® ATMP Bacteria

Microsart® ATMP Fungi

Microsart® ATMP Sterile Release

Workflow bacteria & fungi contamination detection

Microsart® ATMP Extraction

- **DNA isolation using the Microsart® ATMP Extraction kit**
  - Extraction protocol includes centrifugation step to remove free bacterial DNA
  - Harsh extraction allows to isolate Fungi & Bacteria

Microsart® ATMP Bacteria

Microsart® ATMP Fungi

- **2 real-time PCRs using the Microsart® ATMP Fungi & Microsart® ATMP Bacteria kit**
  - Taq-Man® System → reduce false-positive signals
  - Duplex real-time PCR assay → reduce false-negative signals
  - Universal assay for different real-time PCR cycler → FAM™ and ROX™
  - Highly stability & no freezing → Lyophilized reagents

Microsart® ATMP Sterile Release

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Simplifying Progress

Technical background
DNA-based detection methods

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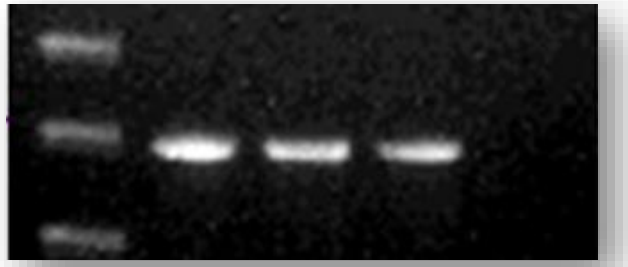
DNA-based detection methods

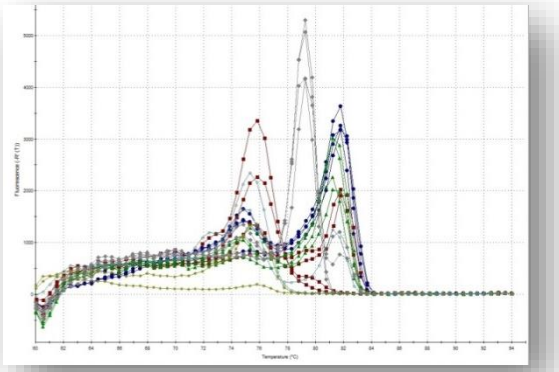
real-time PCR

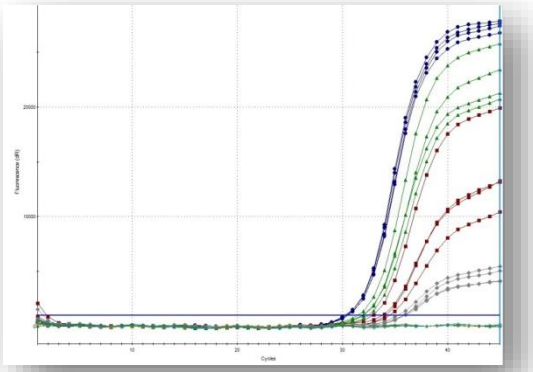
Conventional PCR

SYBR Green I

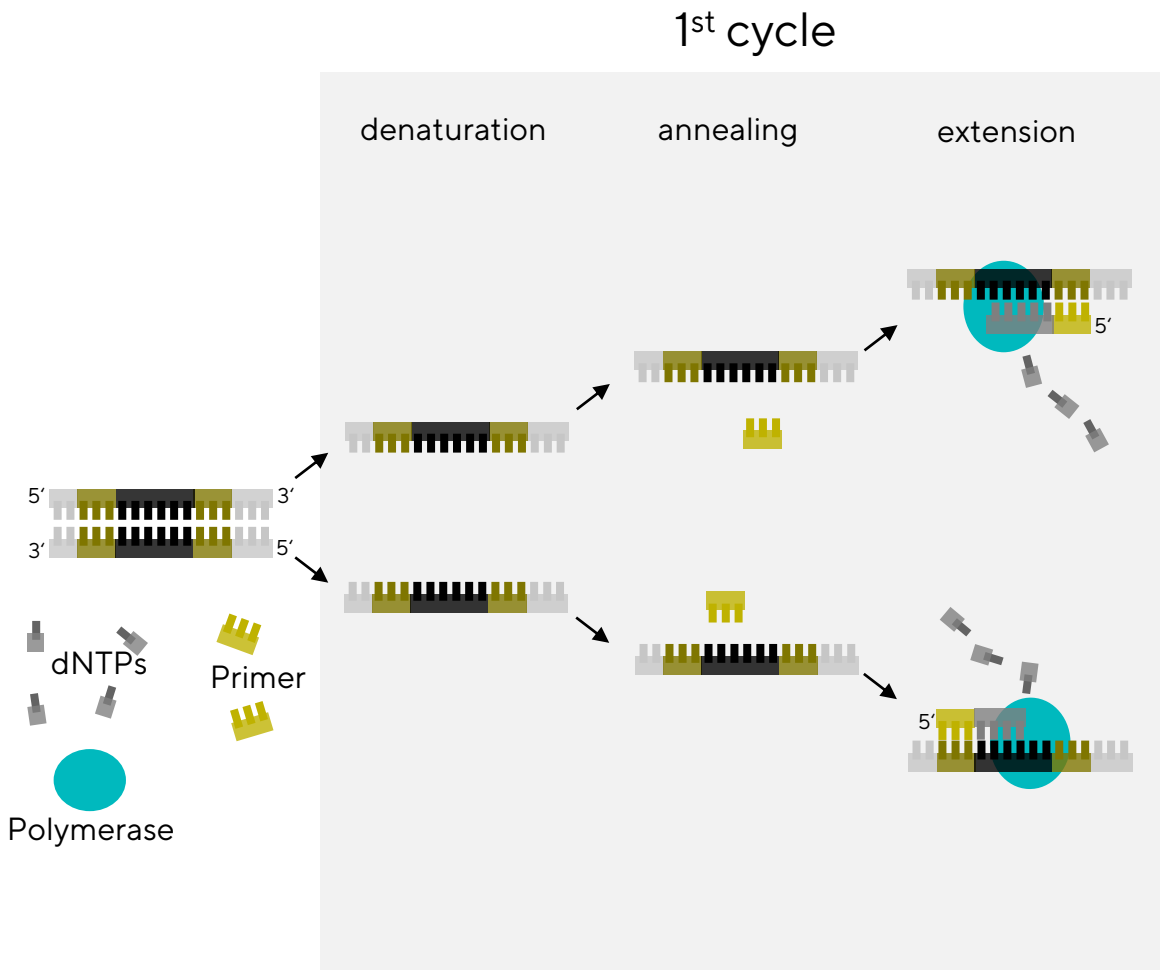
TaqMan® Probe



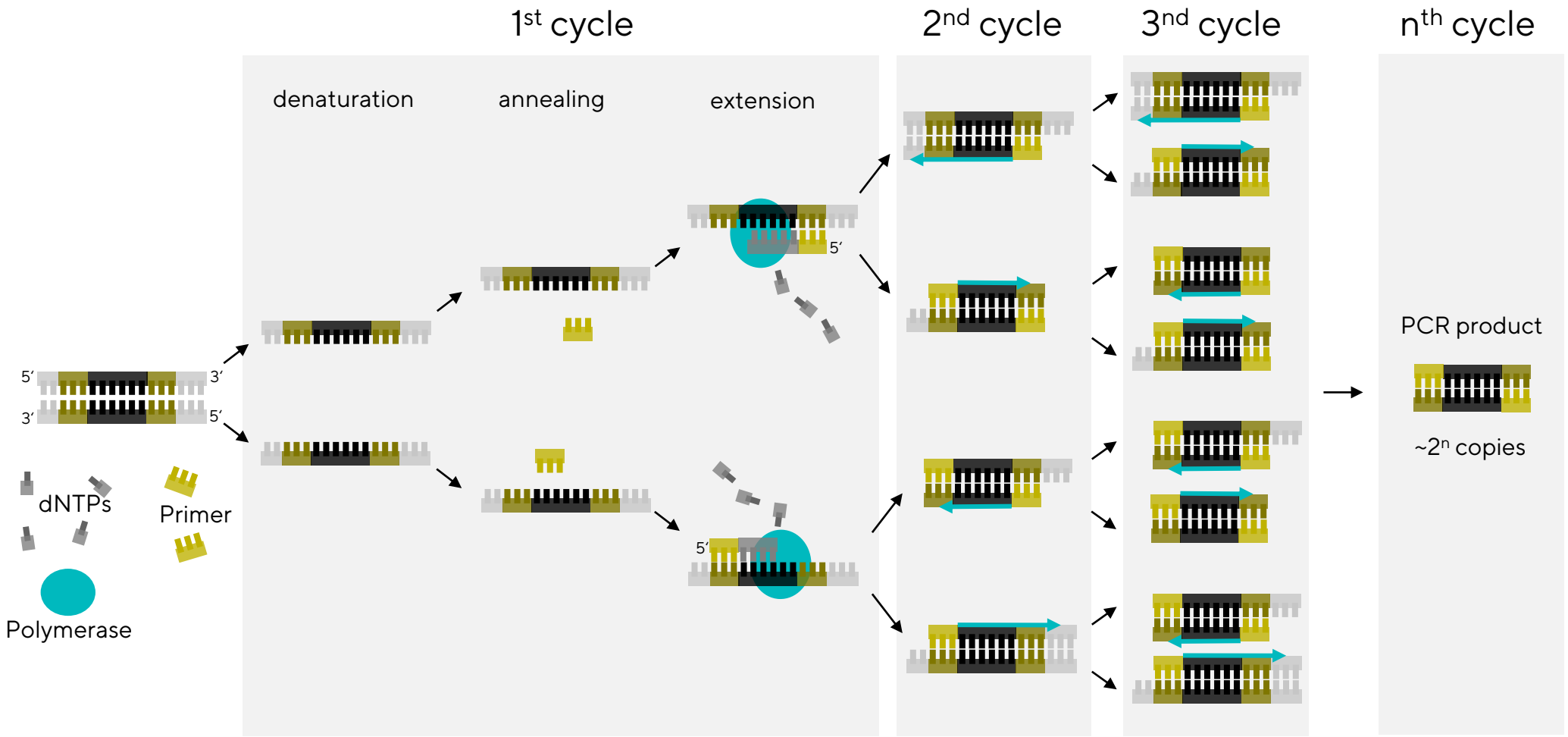




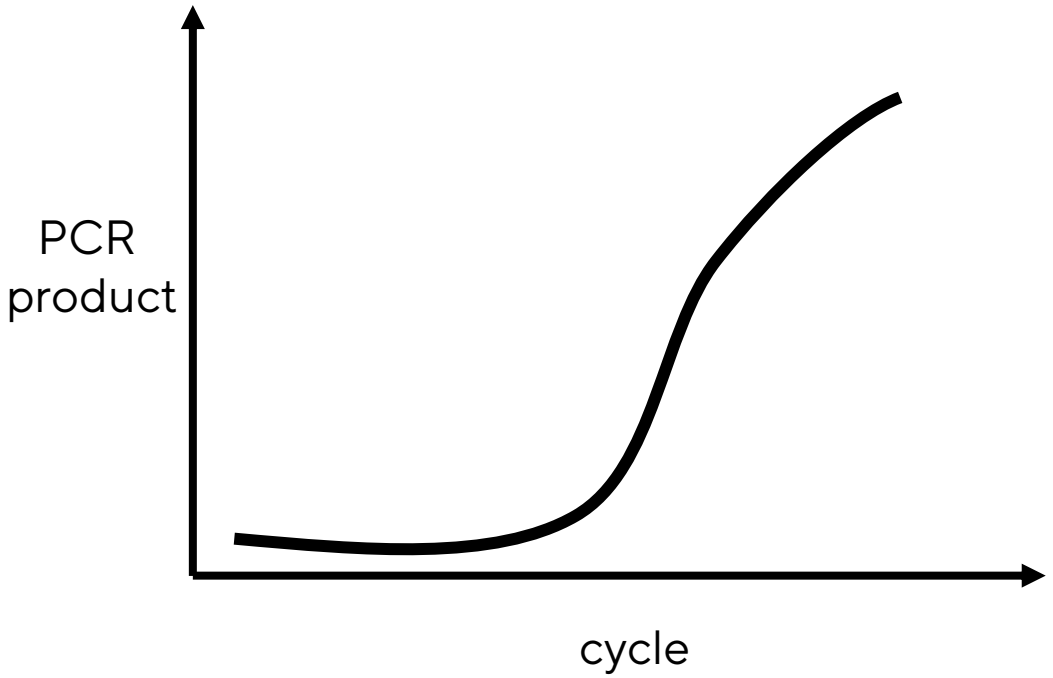
What is a conventional PCR?



What is a conventional PCR?

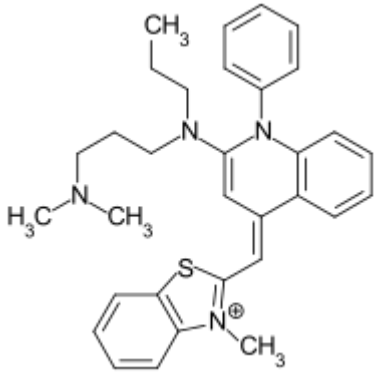


A real-time PCR visualizes the reaction

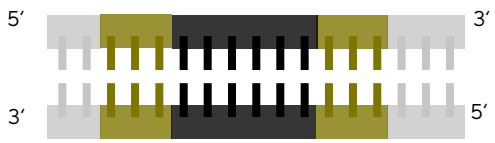


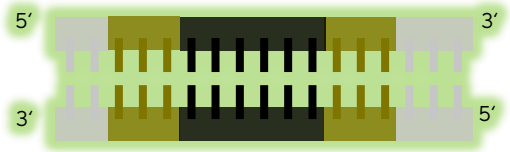
How does that work?

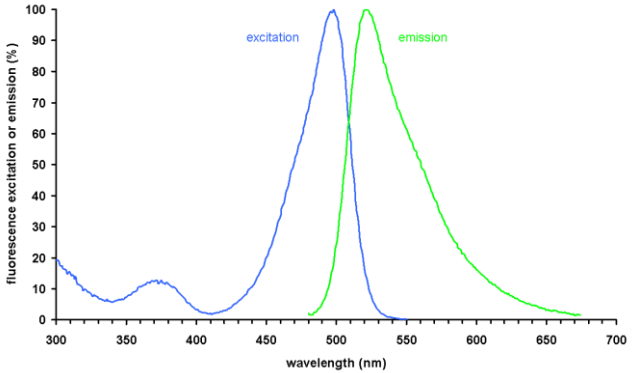
Real-time PCR



SYBR Green I







The dye SYBR Green I binds to double stranded DNA!

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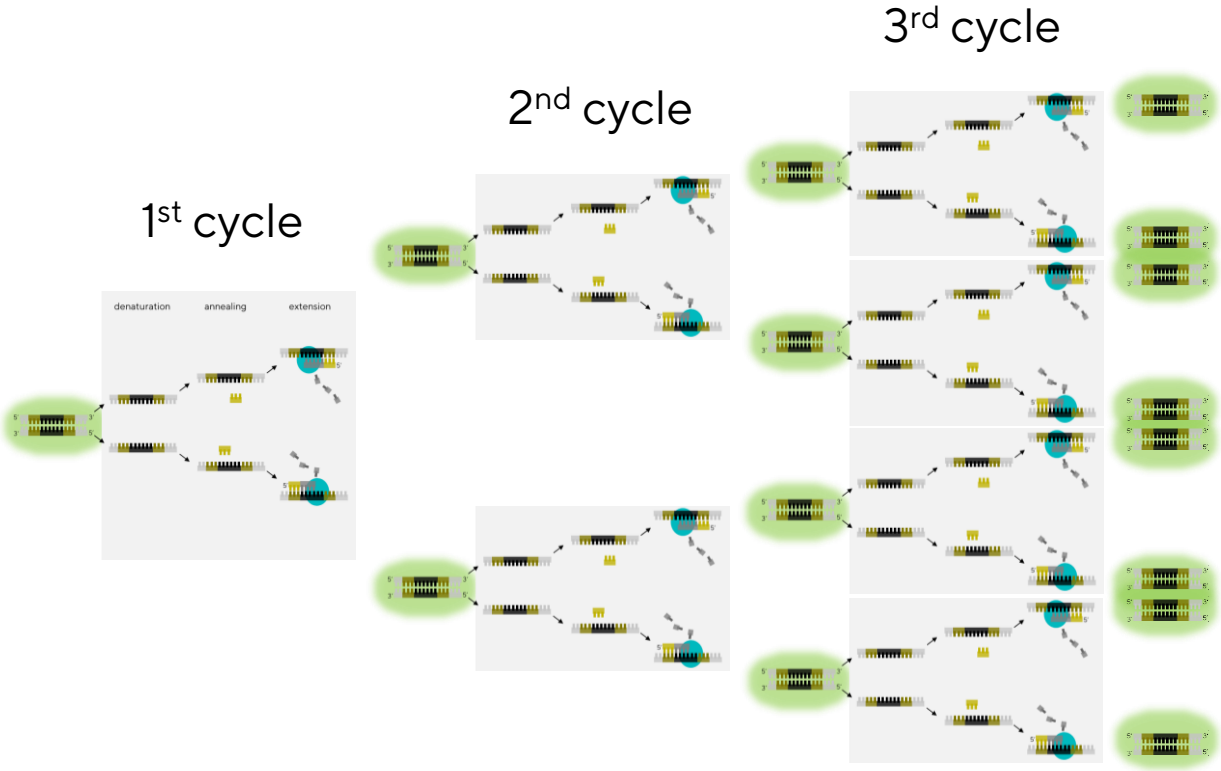
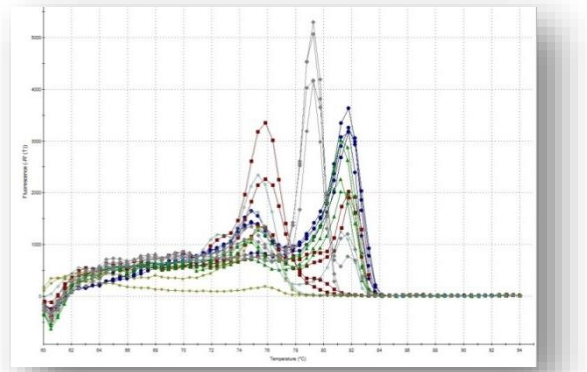
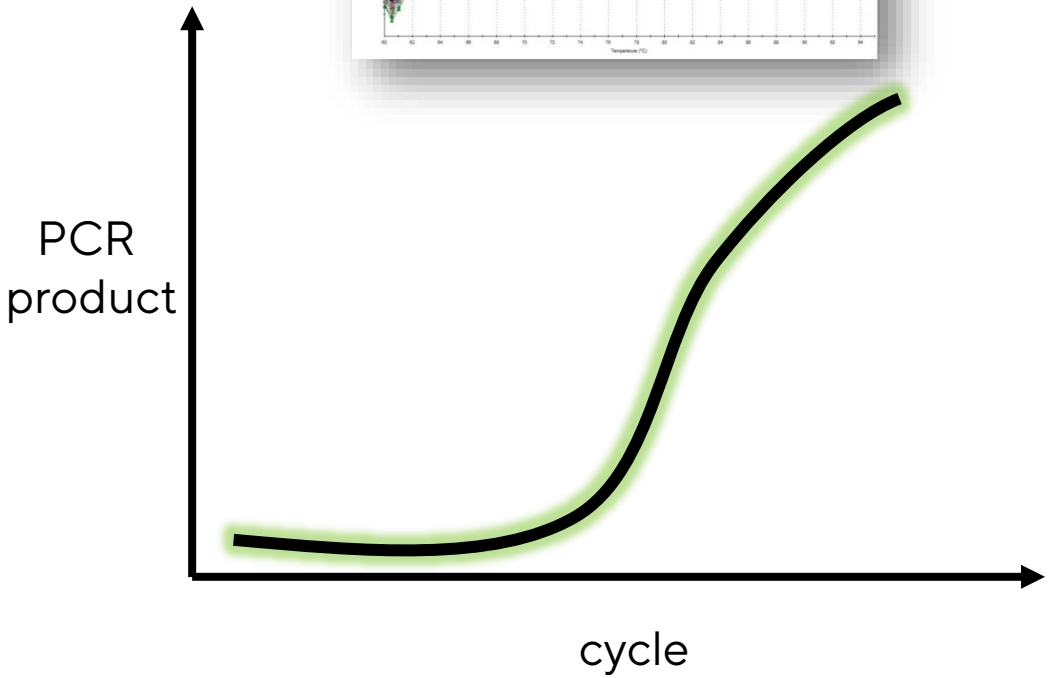
Real-time PCR



SYBR Green I





Unspecific binding of SYBR Green I can result in **false-positive signals!**

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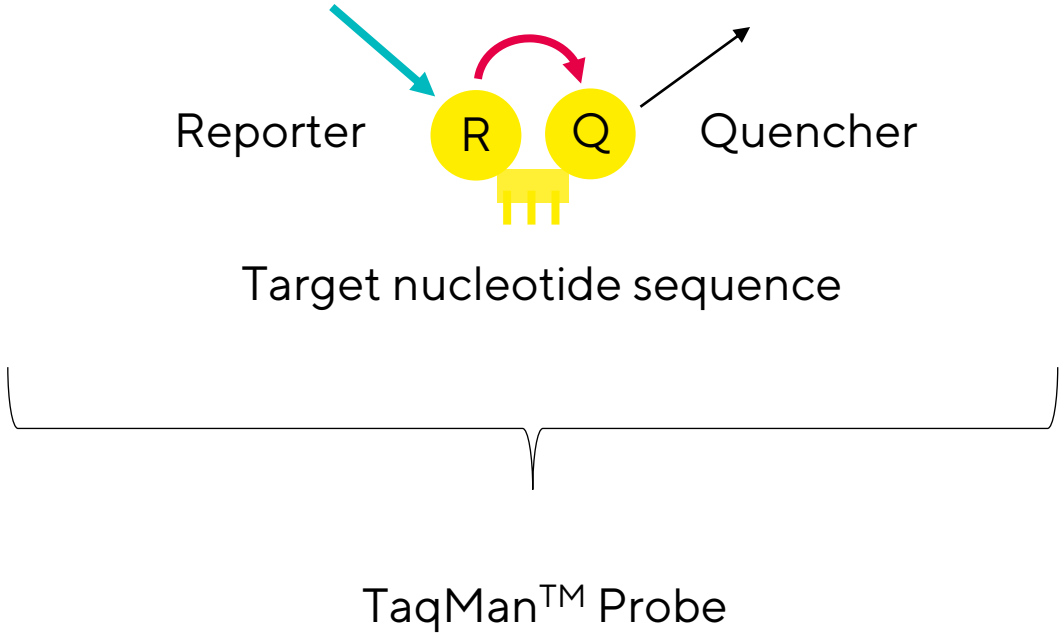
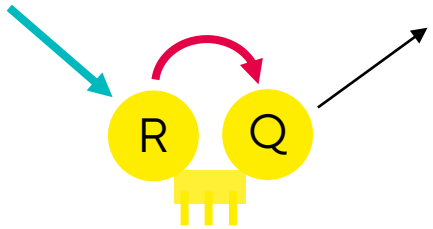
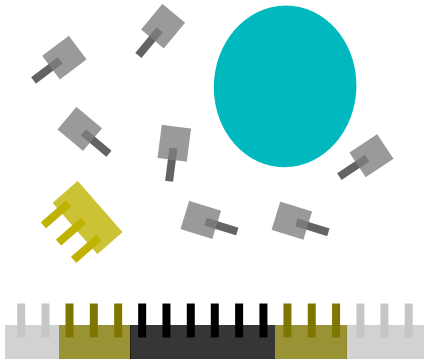
A TaqMan™ probe is more specific compared to SYBR Green I



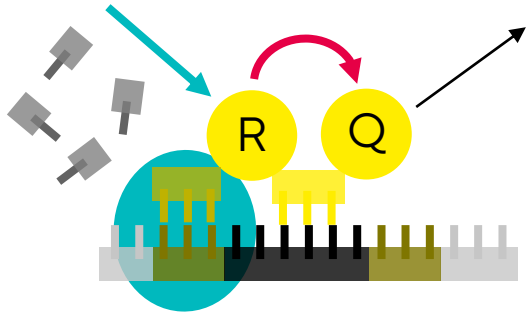
TaqMan™ real-time PCR

TaqMan™ probe is degraded during real-time PCR



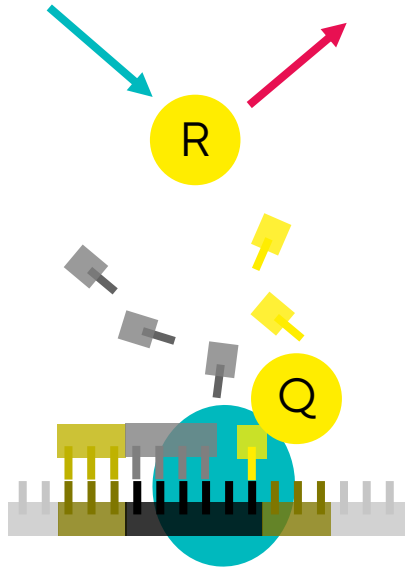


As long as the probe is complete no light signal can be detected



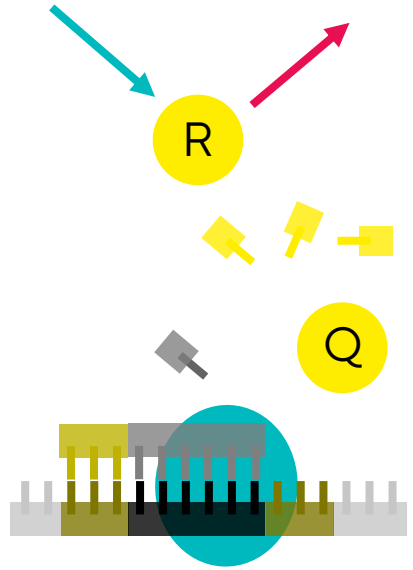
Taq Polymerase functions:

- DNA amplification
- 5’-3’ exonuclease activity



During elongation:

- Polymerase hydrolyses probe
- Dye and quencher are separated
- Reporter dye emits light signal



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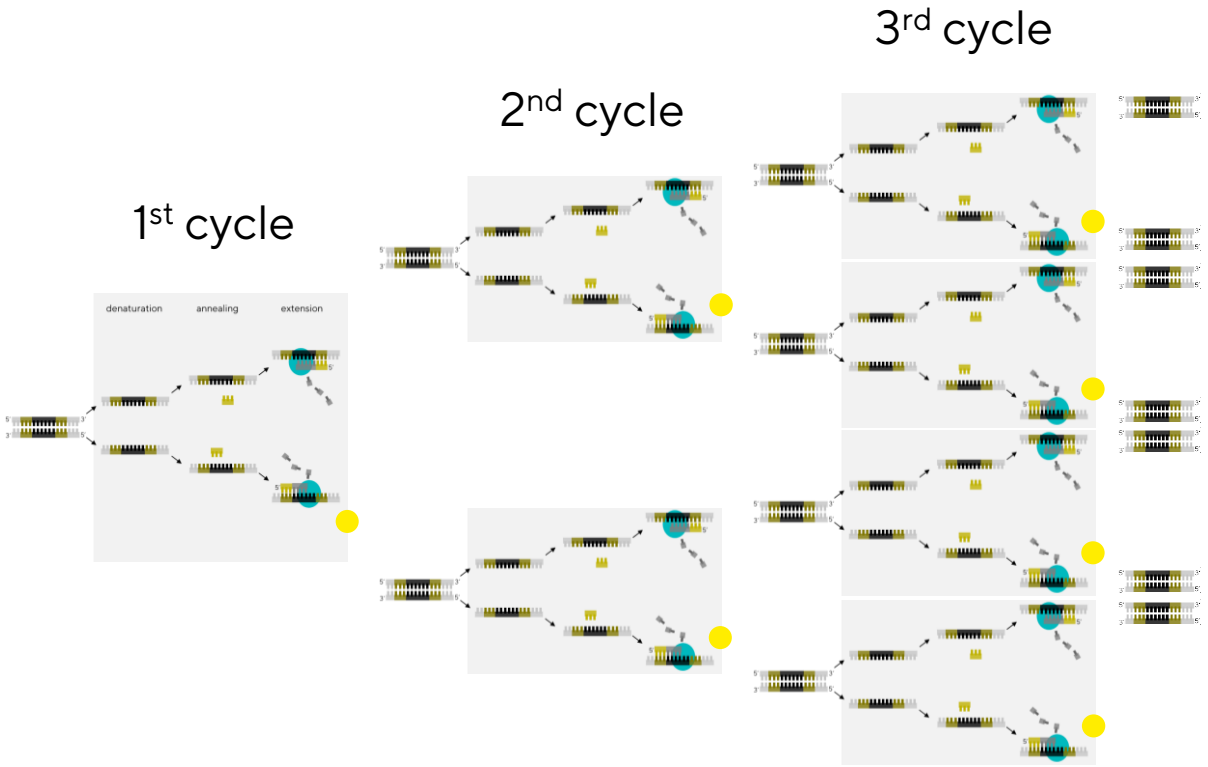
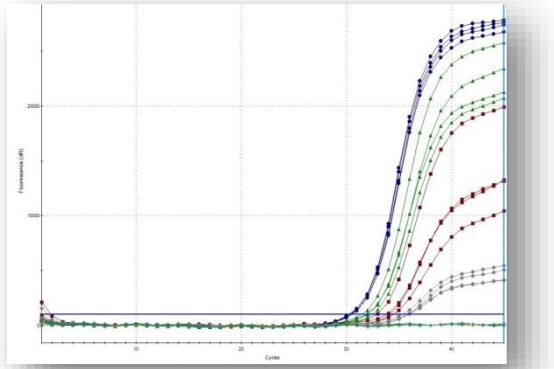
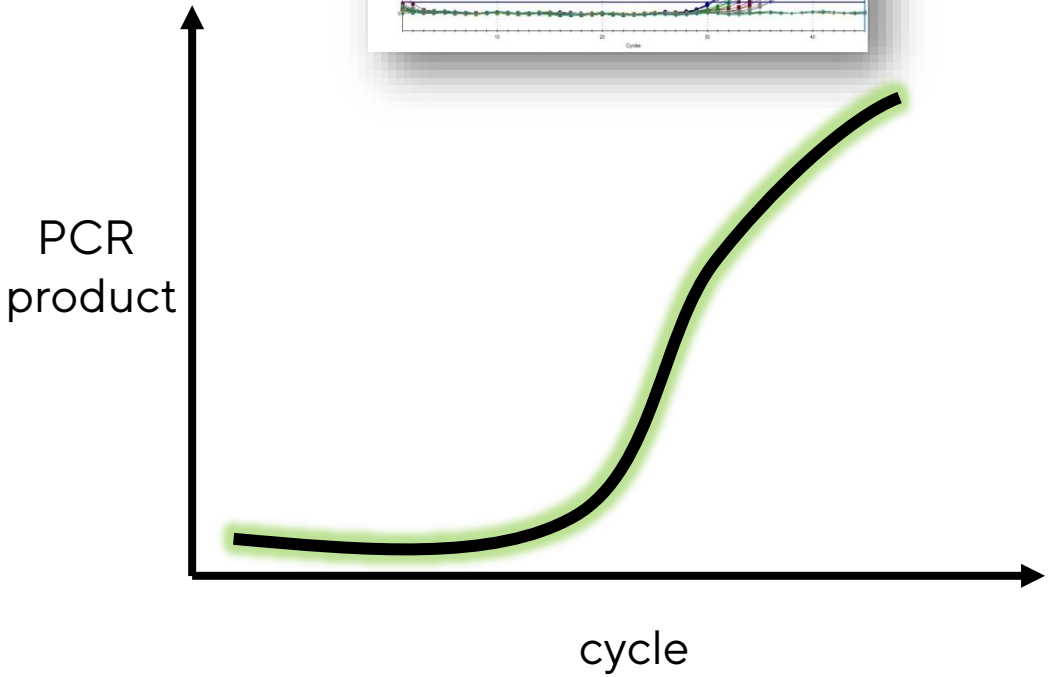
TaqMan™ real-time PCR



TaqMan® Probe





The specificity of TaqMan™ system **reduces false-positive results!**

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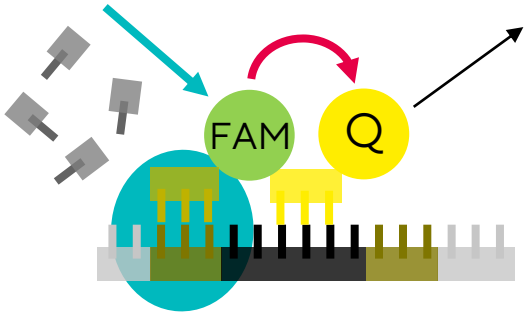
A duplex real-time PCR assay monitors PCR functionality

Problem:
What does **no signal** mean?
No microbial contamination?
PCR inhibition?

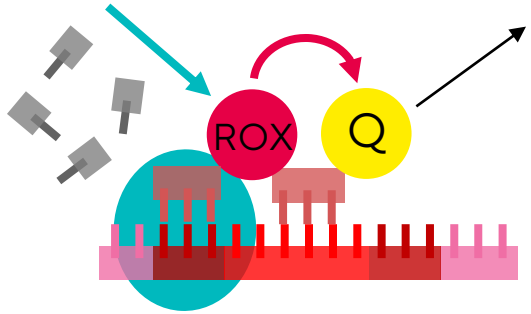
Solution:
Include a second real-time PCR and a control DNA that must lead to a signal!

→ If this internal control reaction does not lead to a signal, the PCR is inhibited.

Duplex assay
= two independent real-time PCRs in one run using different fluorophores



target-probe
target-DNA



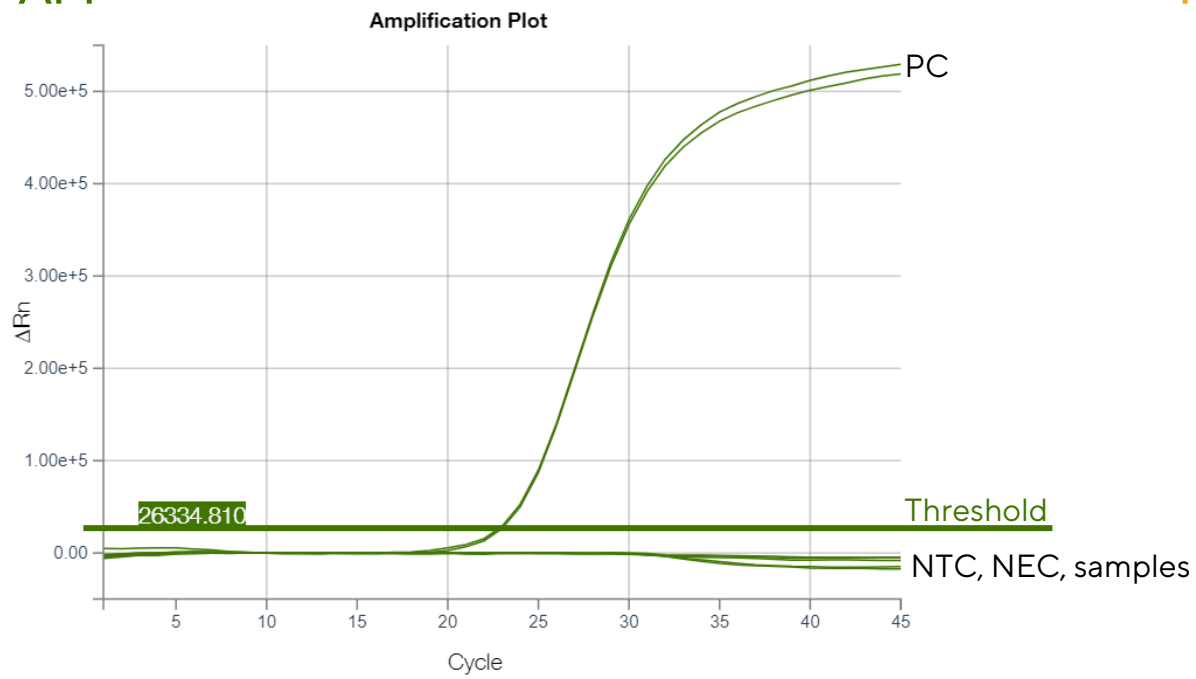
control-probe
control-DNA

The internal control reaction **reduces false-negative results!**

A duplex real-time PCR Analysis

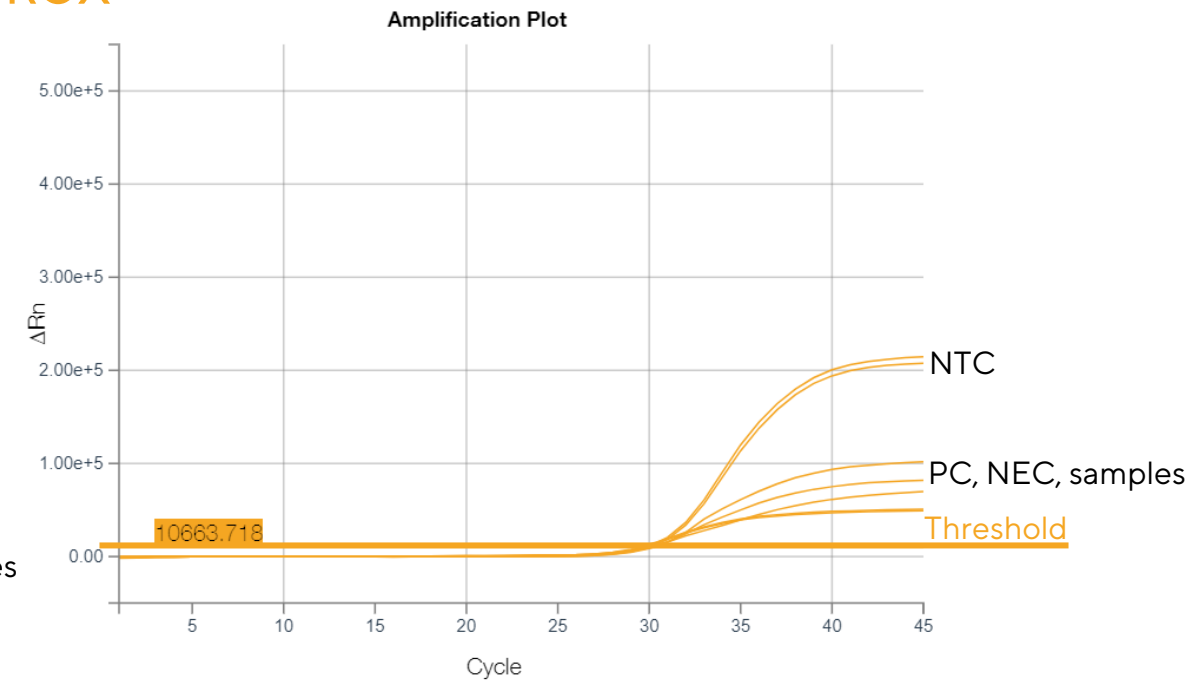
This analysis is done for both, the bacteria real-time PCR and the fungi real-time PCR

FAM



There is no contamination in the samples, because only the positive control is detectable in the FAM channel

ROX



There is no PCR inhibition, because the internal control DNA was detected in all reactions.

What is in the kits?

real-time PCR master mix Bacteria

- Primer for bacterial DNA
- Primer for control DNA
- FAM probe for target DNA
- ROX probe for control DNA
- Taq polymerase
- Buffer

real-time PCR master mix Fungi

- Primer for fungal DNA
- Primer for control DNA
- FAM probe for target DNA
- ROX probe for control DNA
- Taq polymerase
- Buffer

Rehydration buffer
Internal Control DNA
Positive Control DNA
Ultrapure Water

Elution Buffer*
Lysis Buffer*

*Only in Microsart® ATMP Sterile Release

Simplifying Progress

Hands on fungi & bacteria detection

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Microsart® ATMP Extraction

1. Sample Collection

+ 1 ml sample material

Processing tube

15 min
≥ 16,200 × g

Discard supernatant

Store at ≤ -18 °C

or proceed to DNA extraction

2. DNA Extraction

+ 500 µl Lysis Buffer (transparent cap)

⊛

≥ 30 sec vigorously

80 °C, 10 min

≥ 16,200 × g, 10 min

Remove supernatant carefully

+ 100 µl Suspension Buffer (violet cap)

≥ 30 sec vigorously

DNA ready for PCR

Microsart® ATMP Bacteria

1. Rehydration of Reagents

- Bacteria Mix
- Positive Control DNA
- Internal Control DNA

390 µl → Bacteria Mix
800 µl → Internal Control
300 µl → Positive Control

- for 5 min RT
- briefly
- for 5 sec

2. Preparation of PCR Reaction

a) Internal Control added during DNA extraction

+ 15 µl Bacteria Mix (red cap)

* do not forget to add 1 µl Internal Control to NTC and PC

b) Internal Control not added during DNA extraction

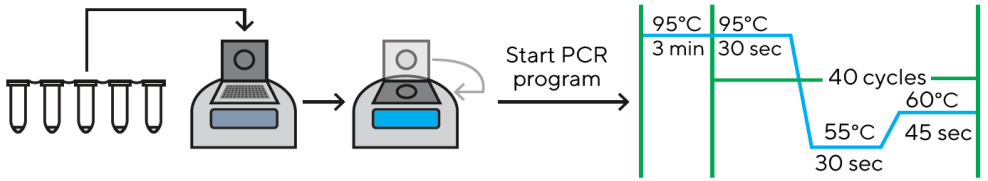
Reaction Mix
+15 µl Bacteria Mix (red cap)
+1 µl Internal Control (yellow cap)

+15 µl Reaction Mix

↓

+ 10 µl sample
+ 10 µl Positive Control (green cap)
+ 10 µl PCR grade Water (white cap)

3. Starting PCR Reaction



- Rehydration Buffer
- Bacteria Mix
- PCR grade Water
- Positive Control
- Internal Control

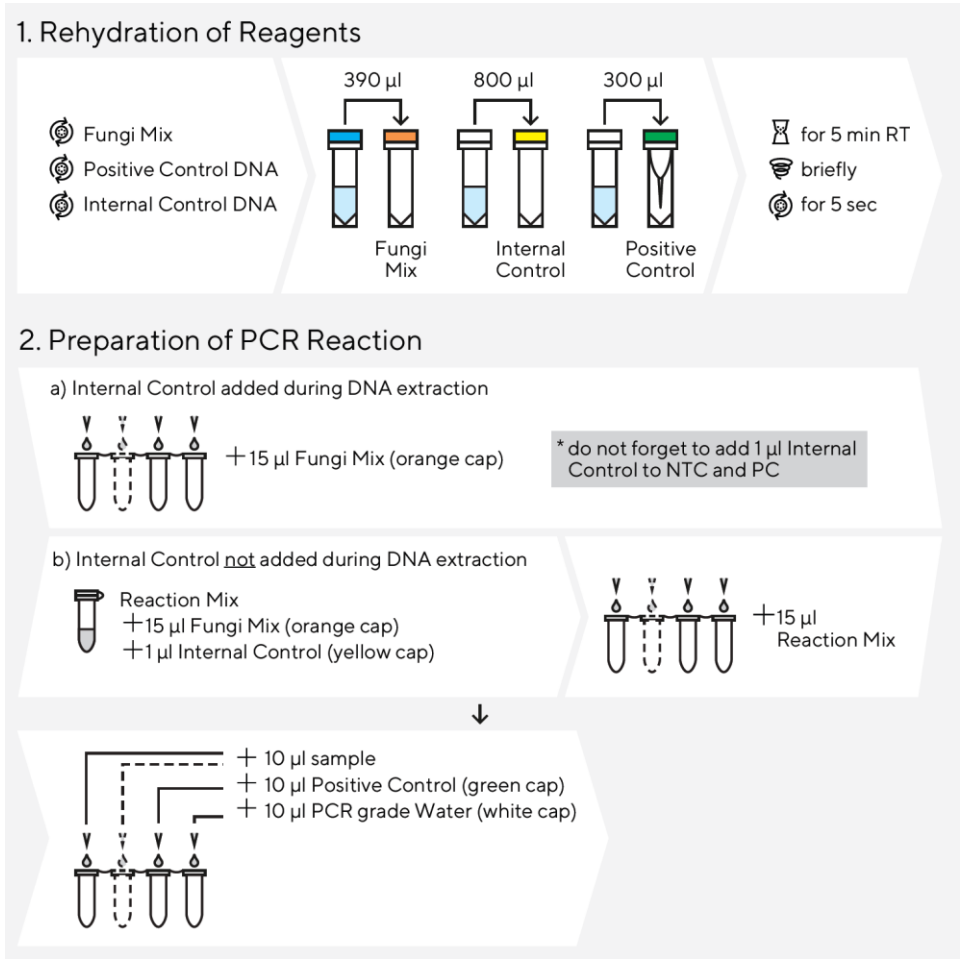
- incubate
- vortex
- centrifuge
- add

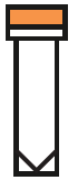
storage +2 - +8 °C
after rehydration ≤ -18 °C

This procedure overview is not a substitute for the detailed manual.

ST_SI_Microsart®-ATMP-Bacteria_04_EN

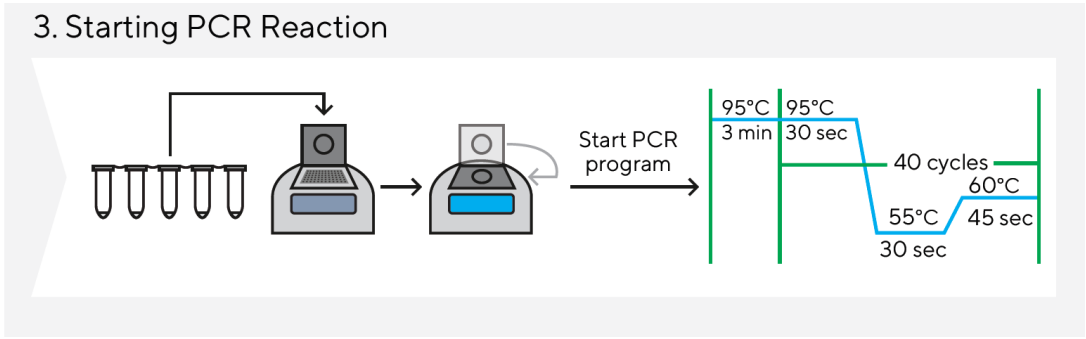
Microsart® ATMP Fungi





Fungi Mix

All identical, but the fungi master mix has an orange cap!



- Rehydration Buffer
- Fungi Mix
- PCR grade Water
- Positive Control
- Internal Control

- incubate
- vortex
- centrifuge
- add

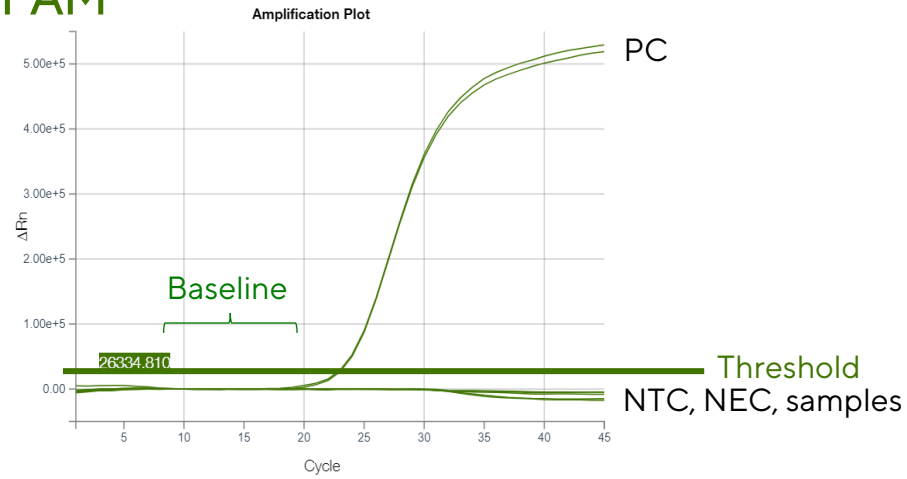
storage +2 - +8 °C
after rehydration ≤ -18 °C

This procedure overview is not a substitute for the detailed manual.

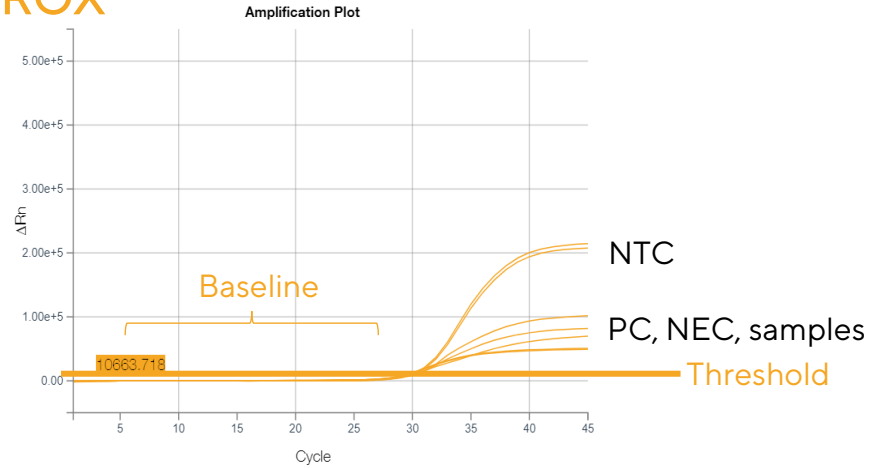
ST_SI_Microsart®-ATMP-Fungi_02_EN

Microsart® ATMP Sterile Release - Analysis

FAM



ROX



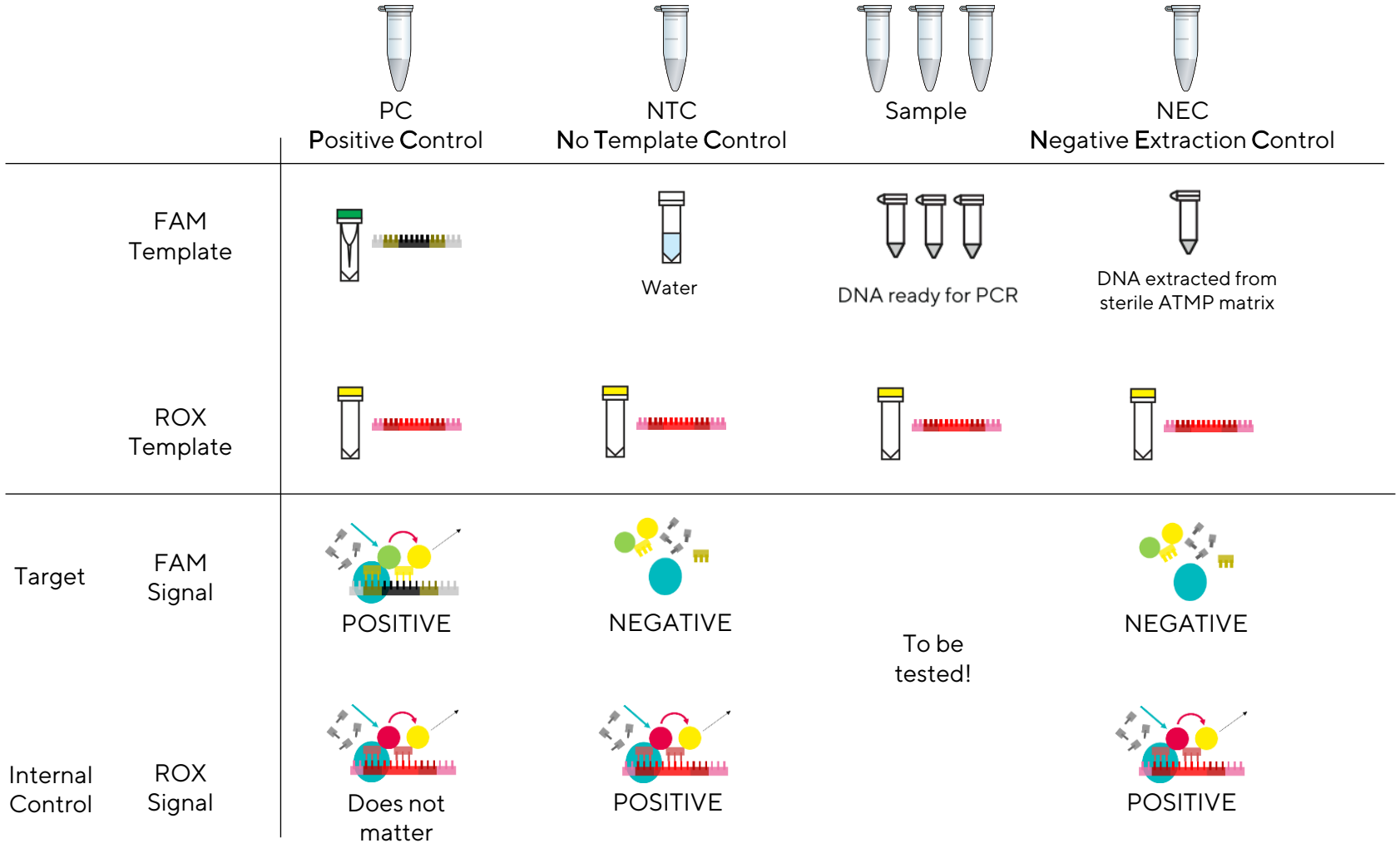
1. Set the baseline to level the curves
2. Set the threshold
3. Check if all controls are as expected

   → see next slide
4. Analyze your samples

Identical analysis workflow for both, the fungi and the bacteria real-time PCR!

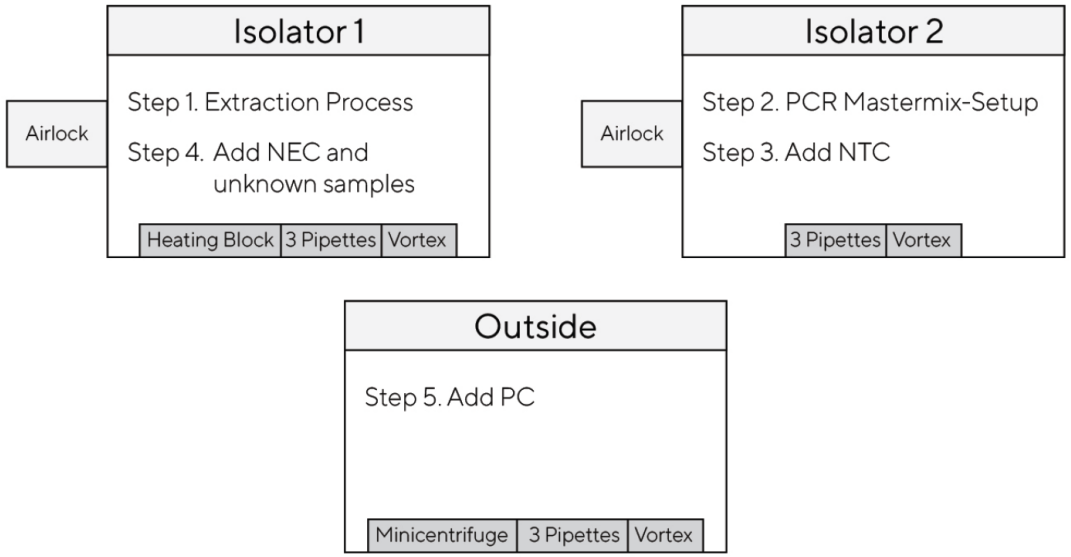
The result interpretation is identical for both, the bacteria real-time PCR and the fungi real-time PCR

Result interpretation

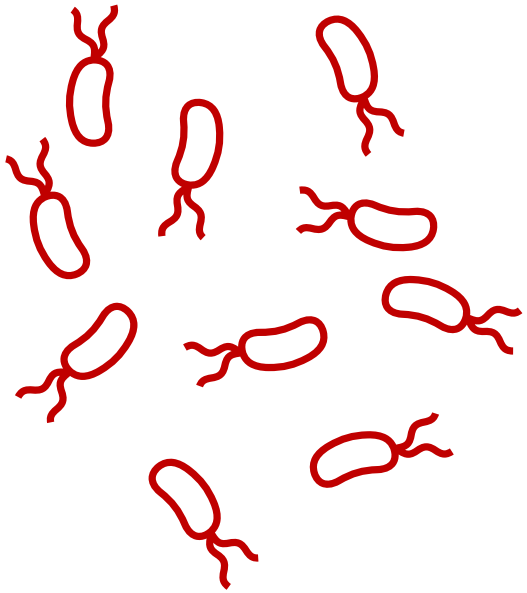


Tips and tricks

- Extensive cleaning with chlorine-based agents
- Avoid cleaning with ethanol → Ethanol precipitates DNA
- Spatial separation of DNA extraction process, master mix setup and positive control
  - E.g. isolator/glovebox or laminar flow
- Work carefully e.g. do not touch the lids of open tubes



Bacterial DNA is everywhere!



Simplifying Progress

Sartorius validation support

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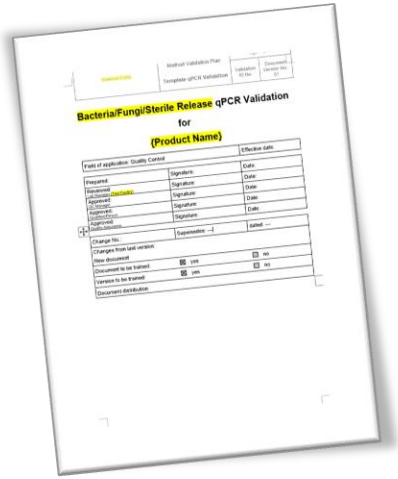
Validation reports, templates & testing proposals



- Product Validation Reports
  - Microsart® ATMP Bacteria + Microsart® ATMP Extraction
  - Microsart® ATMP Fungi + Microsart® ATMP Extraction
- Validation Template
  - Combined validation Template for Microsart® ATMP Bacteria + Microsart® ATMP Fungi + Microsart® ATMP Extraction
- Validation proposal
  - Standard matrix specific validation
  - Individual support

Further support for your validation

- Microsart® Validation Standard (99 CFU/Vial) & Microsart® Calibration Reagents (10^8 GC/Vial for bacteria, 10^6 GC/Vial for fungi)
  - *Bacillus subtilis*
  - *Pseudomonas aeruginosa*
  - *Kocuria rhizophila | Micrococcus luteus*
  - *Clostridium sporogenes*
  - *Bacteroides vulgatus*
  - *Staphylococcus aureus*
  - *Candida albicans*
  - *Aspergillus brasiliensis*
  - *Aspergillus fumigatus*
  - *Penecillium chrysogenum*
  - *Candida glabrata*
  - *Candida krusei*
  - *Candida tropicalis*

Non-viable CFU Standards!

DNA Standards!

Do you miss a species? Let us know!

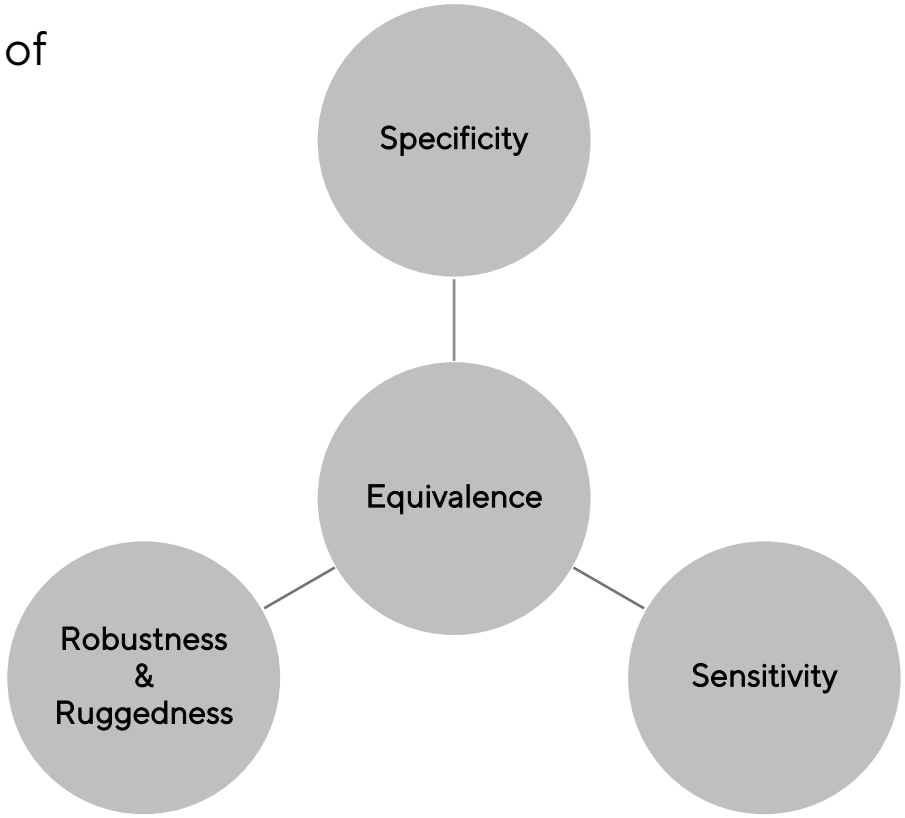
Status quo regulatory landscape

Method	Microbiological QC-Release testing		
	Mycoplasma	Bacteria	Fungi
Classical testing	USP<63> | EP 2.6.7 28 days	USP<71> | EP 2.6.1 Sterility testing 14 days	
real-time PCR-based	EP 2.6.7 (USP<1223>/EP 5.1.6)	USP<1071> EP 2.6.27 (USP<1223>/EP 5.1.6)	

Validation overview

Regulatory guidance for validation of rapid / alternativ methods:

- PDA, TR 33
- USP <1223>
- USP <1071>
- EP 5.1.6 part 4-1-1 primary validation by supplier
- EP 2.6.27
- (USP<71>)
- (EP2.6.1)



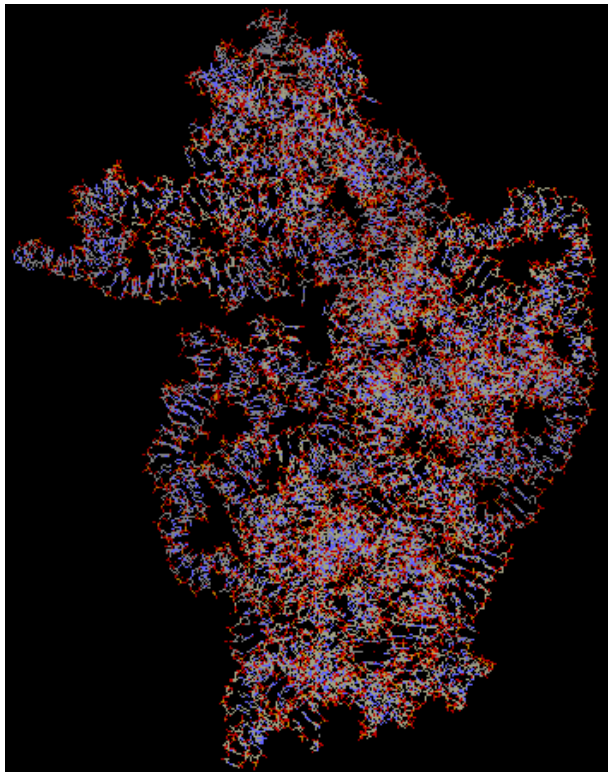
NOT a replacement for the classical sterility test, but valuable addition for patient safety

Including Guidance of the German Governmental Regulatory Agency (part of EMEA)

Paul-Ehrlich-Institut

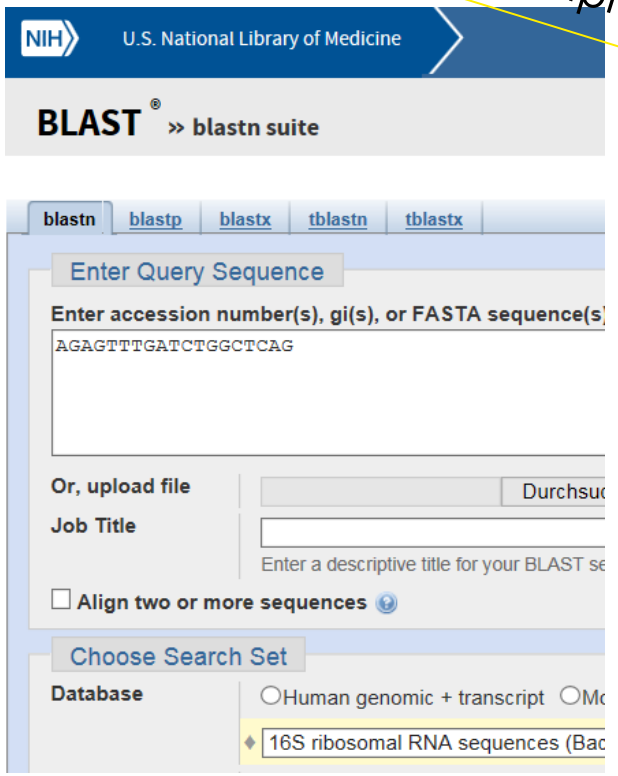
In silico prediction by sequence alignment and blast

16S / 18S rDNA



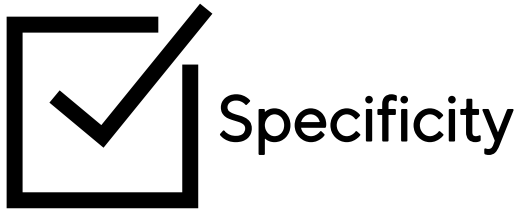
http://www.biochem.umd.edu/biochem/kahn/bchm465-01/ribosome/16SrRNA.html

example



https://www.ncbi.nlm.nih.gov/

Detection range – Microsart® ATMP Bacteria



Species	Strain No	Primer Mismatches		
		Forward primer	Probe	Reverse primer
Bacillus subtilis	ATCC 6633	0	0	0
Clostridium sporogenes	ATCC 3584	0	0	0
Pseudomonas aeruginosa	ATCC 9027	0	0	0
Staphylococcus aureus	ATCC 6538	0	0	0

	Primer Mismatches			
	0	1	2	3
Bacteria	48.8 %	69.4 %	85.7 %	94.7 %
Archaea	n.a	n.a	0.1%	40.4 %
Eukaryotes	0%	0%	0.1%	0.3 %

Accepting 3 primer mismatches, **94.7 % of the bacteria** are detected

Detection range – Microsart® ATMP Fungi

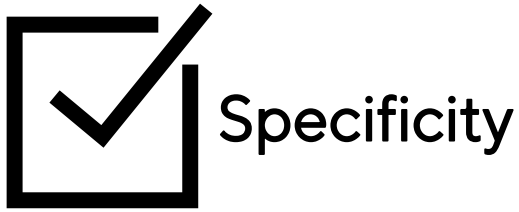
Specificity

Accepting 2 primer mismatches, already **37 % of the fungi** are detected, including all species of clinical and bioprocess relevance.

Genus	Coverage	
Alternaria	97.7 %	
Aspergillus	95.3 %=	
Aureobasidium	93.5 %	
Bipolaris	98 %	
Candida	86.3 %	
Chaetomium	3.6%	← soil, air, plant debris
Cladosporium	95.5 %	
Curvularia	100 %	
Epidermophyton	100 %	
Exserohilum	97.4 %	
Fusarium	95.9 %	
Memnoniella (Stachybotrys)	86,7 %	
Microsporum	100 %	
Myrothecium	100 %	
Paecilomyces	100%	
Penicillium	98.2 %	
*Malassezia**	0.1 %	← skin microbiome
Rhizopus	4 %	← organic substances
Scopulariopsis	0 %	← soil, decaying wood
Trichoderma	98 %	
Trichophyton	100 %	

Species	Strain No	Primer Mismatches		
		Forward primer	Probe	Reverse primer
Aspergillus brasilliensis	ATCC 6275	0	0	0
Candida albicans	ATCC 18804	0	0	0

Matrix Effects



Bacteria

		Results
Hela	No C_q	0/2
	No C_q	
Vero	No C_q	0/2
	No C_q	
CHO-K1	No C_q	0/2
	No C_q	
RK13	No C_q	0/2
	No C_q	
CHO-DG44	No C_q	0/2
	No C_q	
CHO XM111-10	No C_q	0/2
	No C_q	
L-9296 (NCTC)	No C_q	0/2
	No C_q	

Fungi

			Results
Hela	No Cq	No Cq	0/8
	No Cq	No Cq	
	No Cq	No Cq	
	No Cq	No Cq	
Vero	No Cq	No Cq	0/8
	No Cq	No Cq	
	No Cq	No Cq	
	No Cq	No Cq	
CHO-K1	No Cq	No Cq	0/8
	No Cq	No Cq	
	No Cq	No Cq	
	No Cq	No Cq	
HPBMC	No Cq	No Cq	0/8
	No Cq	No Cq	
	No Cq	No Cq	
	No Cq	No Cq	
Jurkat	No Cq	No Cq	0/8
	No Cq	No Cq	
	No Cq	No Cq	
	No Cq	No Cq	

For the tested matrices, **no matrix effects** were detected.

Limit of detection – Microsart® ATMP Bacteria/Fungi

EP 2.6.1
USP<71>

Currently tested = 6

Bacillus subtilis
Clostridium sporogenes
Pseudomonas aeruginosa
Staphylococcus aureus

Candida albicans
Aspergillus brasiliensis

23/24 positive

regulatory advice;
EP 2.6.27; user feedback

Recommended Extension = 20

\+

Colony Forming Units (CFU)

Streptococcus pyogenes
Bacteroides vulgatus
Escherichia coli
Pseudomonas protegens
Bacillus cereus
Enterococcus faecalis
Kocuria rhizophila
Staphylococcus epidermidis
Serratia marcescens
Cutibacterium acnes

Candida tropicalis
Candida glabrata
Candida krusei
Aspergillus fumigatus
Penicillium chrysogenum

Genome Copies (GC)

Bacteroides fragilis
Enterobacter cloacae
Klebsiella pneumoniae
Clostridium perfringens
Yersinia enterocolitica

8/8 positive

Limit of Detection (LOD_{95}): 99, 50, 25, 10, 5, 2.5, 1.25 CFU/ml

Limit of detection

Sensitivity

Example: *Candida albicans*

CFU/ml						Mean	
99	Run 1	31.32	31.90	32.78	32.80	32.20	24/24
	Run 2	32.96	32.88	33.07	32.25	32.79	
	Run 3	32.29	32.81	36.03	32.09	33.30	
	Run 4	32.46	32.14	32.29	32.33	32.30	
	Run 5	32.32	32.55	32.71	32.36	34.48	
	Run 6	32.58	31.48	31.89	32.43	32.09	
50	Run 1	33.15	33.79	34.11	34.44	33.87	23/24
	Run 2	34.25	33.86	33.04	35.03	34.04	
	Run 3	33.59	33.90	32.63	33.89	33.50	
	Run 4	34.04	32.94	34.27	33.81	33.76	
	Run 5	No Cq	34.77	32.82	34.48	34.02	
	Run 6	32.58	32.57	32.82	34.81	31.19	
25	Run 1	33.42	34.72	34.88	35.32	34.58	21/24
	Run 2	34.67	37.19	33.28	36.08	35.30	
	Run 3	34.96	37.20	38.18	No Cq	36.78	
	Run 4	37.96	34.90	35.11	34.59	35.64	
	Run 5	33.55	No Cq	35.77	No Cq	34.66	
	Run 6	34.51	35.33	36.17	34.90	35.23	
10	Run 1	35.85	36.48	37.27	37.38	36.74	14/24
	Run 2	No Cq	No Cq	37.00	34.65	35.82	
	Run 3	No Cq	No Cq	No Cq	No Cq	No Cq	
	Run 4	No Cq	35.93	No Cq	37.11	36.52	
	Run 5	35.04	No Cq	No Cq	38.91	36.97	
	Run 6	35.54	36.26	35.84	37.52	36.29	

currently tested

recommended extension

Species	Strain No	Acceptance criterion	LOD_{95} (CFU/mL)
Candida albicans	ATCC 10231	23/24	50
Aspergillus brasiliensis	ATCC 16404	23/24	50
Candida tropicalis	ATCC 750	8/8	10
Candida glabrata	ATCC 90030	8/8	25
Candida krusei	ATCC 6258	8/8	50
Aspergillus fumigatus	ATCC 9197	8/8	99
Penicillium chrysogenum	ATCC 9178	8/8	99

Limit of detection

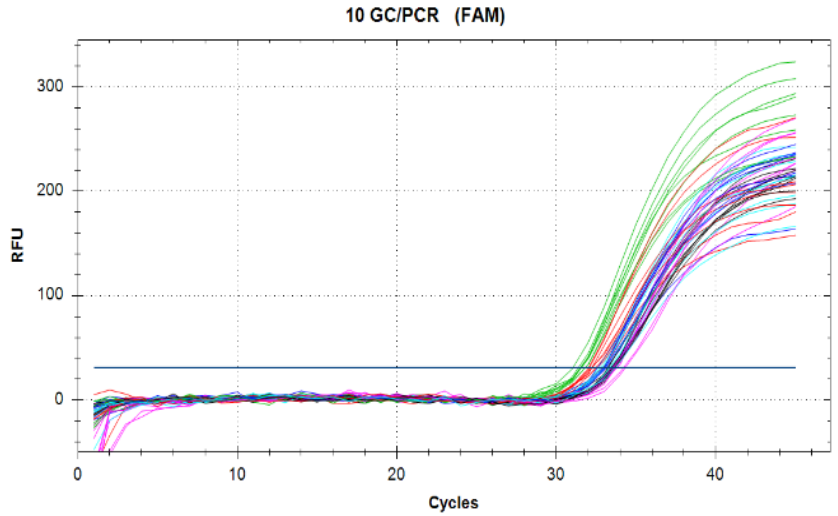
Sensitivity

Species	Strain No	Acceptance criterion	LOD_{95} (CFU/mL)
Bacillus subtilis	ATCC 6633	23/24	25
Clostridium sporogenes	ATCC 19404	23/24	25
Pseudomonas aeruginosa	ATCC 9027	23/24	5
Staphylococcus aureus	ATCC 6538	23/24	10
Streptococcus pyogenes	ATCC 19615	8/8	99
Bacterioides vulgatus	ATCC 8482	8/8	2,5
Escherichia coli	ATCC 8739	8/8	10
Pseudomonas protegens	ATCC 17386	8/8	10
Bacillus cereus	ATCC 10876	8/8	5
Enterococcus faecalis	ATCC 29212	8/8	99
Kocuria rhizophila	ATCC 9341	8/8	10
Staphylococcus epidermidis	ATCC 12228	8/8	99
Serratia marcescens	ATCC 14756	8/8	50
Propionibacterium acnes	ATCC 11827	8/8	25

currently tested

recommended extension

Species	Strain No	Acceptance criterion	LOD_{95} (GC/PCR)
Bacteroides fragilis	ATCC 25285	8/8	10
Enterobacter cloacae	ATCC 13047	8/8	10
Klebsiella pneumoniae	ATCC 13883	8/8	10
Serratia marcescens	ATCC 13880	8/8	10
Clostridium perfringens	ATCC 13124	8/8	10
Yersinia enterocolitica	ATCC 27739	8/8	10



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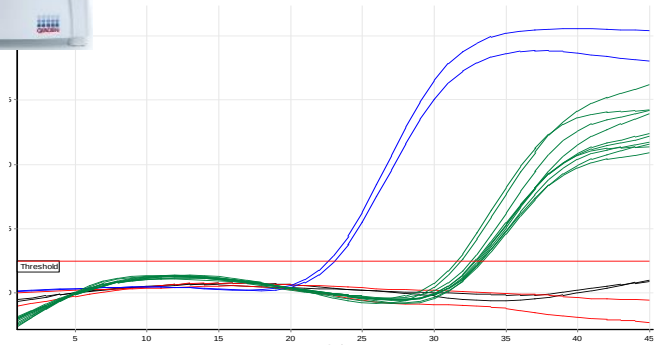
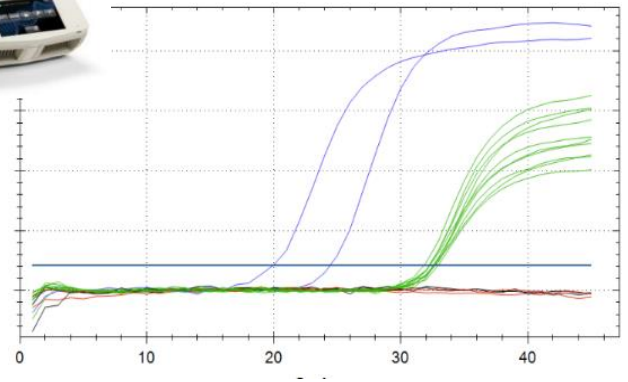
Device comparability

Robustness & Ruggedness

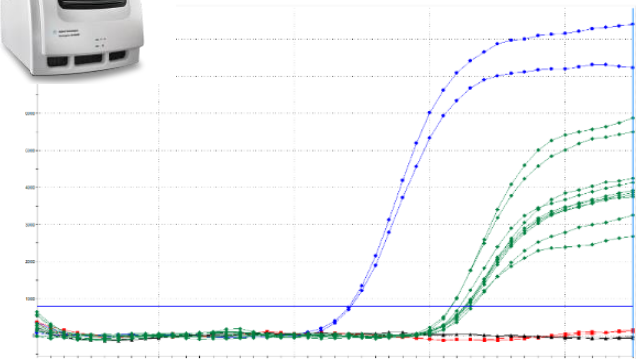
Qiagen Rotor-Gene 6000



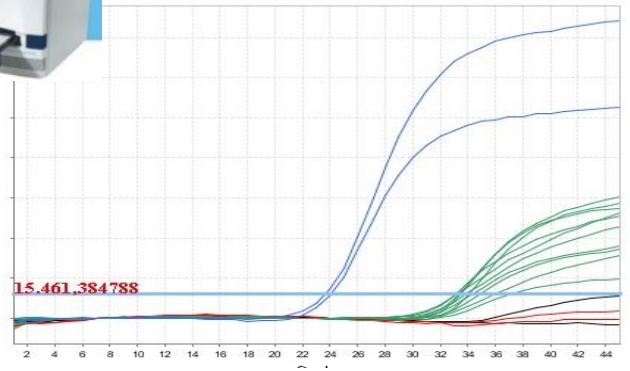
BioRad CFX96 touch



Agilent Mx3005p



Thermo Fischer ABI Prism 7500



Spiking 99 CFU/ml of the species with the highest LOD_{95}

- *Clostridium sporogenes* (LOD_{95} = 25)
- *Candida albicans* (LOD_{95} = 50)

Acceptance criterion 8/8 positive

PC
NTC
NEC
Extracts

… by today many more devices are used by our customers.

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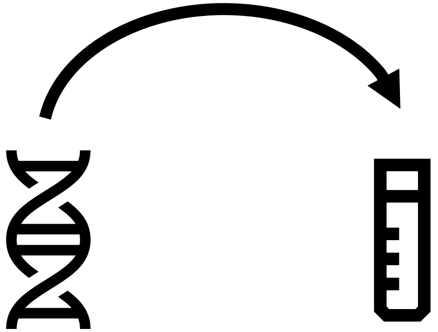
False-positives due to free microbial DNA?

Robustness & Ruggedness



E. coli DNA spiked into DMEM
↓
DNA extraction
↓
Real-time PCR

failed		passed							
10^4 GC/mL		10^3 GC/mL		100 GC/mL		10 GC/mL		0 GC/mL	
Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
39.00	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q
No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q
39.93	39.89	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q
39.38	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q
No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q
No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q
No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q
No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q	No C_q

There is a low risk of false-positives, because the free DNA is removed efficiently.

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False-positives due to cell culture medium?

Robustness & Ruggedness

	Bacteria		Fungi	
	Negative Results	% Negative results	Negative Results	% Negative results
DMEM + 5% FCS	8/8	98,95 %	8/8	100 %
	8/8		8/8	
	8/8		8/8	
	8/8		8/8	
	8/8		8/8	
	7/8		8/8	
	8/8		8/8	
	8/8		8/8	
	8/8		8/8	
	8/8		8/8	
	8/8		8/8	
	8/8		8/8	
DMEM	8/8	100 %	8/8	100 %
	103/104	99 %	104/104	100%

DMEM* without spike acceptance criterion > 95 % negative

* from Biochrom AG

There is a low risk of false-positives, because cell culture medium is DNA-free.

False-positives due to cell culture medium?

Robustness & Ruggedness

Fungi detection	Negative Results	ROX™	% Negative results
DMEM	8/8	8/8 Correct	100%
Fetal bovine serum (FBS)	8/8	8/8 Correct	100%
DMEM high Glucose, GlutaMAX	8/8	8/8 Correct	100%
DMEM w/o Na-Pyruvat mit stable Glutamin	8/8	8/8 Correct	100%
RPMI 1640	8/8	8/8 Correct	100%
MEM (1)	8/8	8/8 Correct	100%
MEM (2)	8/8	8/8 Correct	100%
KnockOut DMEM	8/8	8/8 Correct	100%
DMEM/F-12 GlutaMAX	8/8	8/8 Correct	100%
Opti-MEM Reduced Serum GlutaMAX	8/8	8/8 Correct	100%
McCoys 5A Medium	8/8	8/8 Correct	100%
Leibovitz L-15 Medium	8/8	8/8 Correct	100%
Chrondrocyte Differentiation Medium	8/8	8/8 Correct	100%
Human Osteoblast Differentiation Medium	8/8	8/8 Correct	100%
ChondroMAX Differentiation Medium	8/8	8/8 Correct	100%
Tahara Lympho One + HS	8/8	8/8 Correct	100%
RPMI, penicillin/Streptomycin, Glutamax, 5% FBS	8/8	8/8 Correct	100%

Cell culture medium without spike acceptance criterion > 95 % negative

There is a low risk of false-positives, because cell culture medium is DNA-free.

Tolerance of eukaryotic cell background

Robustness & Ruggedness

	Aspergillus brasiliensis 99 CFU (FAM™)			
	10^6 cells/ml		10^5 cells/ml	
Hela	37.54	31.26	33.72	32.10
	35.24	31.84	33.34	32.07
	4/4		**4/4**	
Vero	33.30	31.43	32.78	31.39
	34.45	32.76	33.48	31.60
	4/4		**4/4**	
CHO-K1	33.65	32.40	34.49	33.36
	34.70	32.83	33.89	32.40
	4/4		**4/4**	
HPBMC	32.21	31.59	33.80	32.12
	32.39	31.25	33.79	31.04
	4/4		**4/4**	
Jurkat	34.99	34.14	31.77	32.64
	34.80	33.50	34.30	33.81
	4/4		**4/4**	

All organisms were successfully detected in cell backgrounds of 10^5-10^6 cells/ml

Application Notes

Rapid, real-time PCR-based detection of microbial contaminations in high cell density Jurkat-, HPBMC-and CHO-cultures using Microsart® ATMP kits

In this study, we assessed the detection capability of Microsart® ATMP Extraction, combined with Microsart® ATMP Bacteria/Fungi/Mycoplasma assays, in high-density cell cultures.


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Application No

Rapid, Real-Time PCR-Based Detection of Microbial Contaminations in High Cell Density Jurkat-, HPBMC- and CHO- Cultures using Microsart® ATMP Kits

Abstract

Detection limits in different cell types

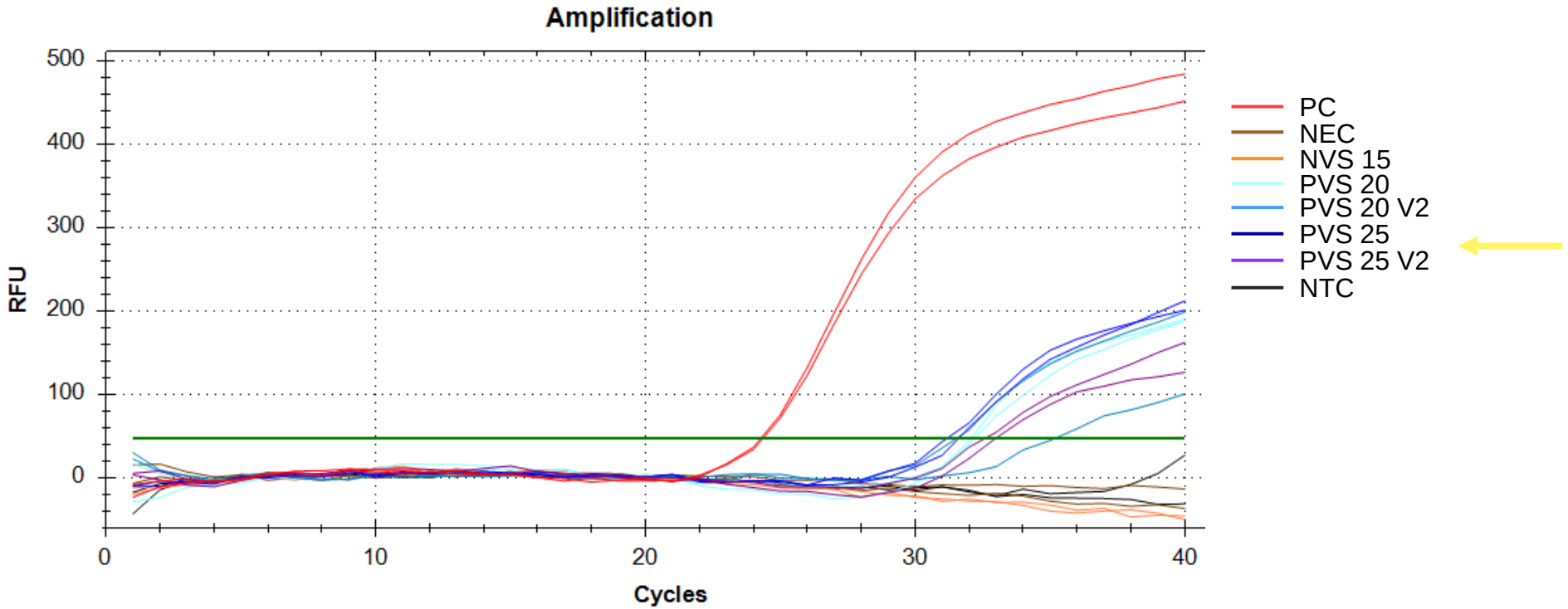
Cell Type Background	Microorganism Spike	Background Cells /mL (In 10^6)	Detection
CHO	99 CFU *B. subtilis*	19.0	Successful
CHO	10 CFU *M. arginini*	15.0 and 15.6 (two individual experiments)	Successful
CHO	10 CFU *M. orale*	15.5 and 16.3 (two individual experiments)	Successful
Jurkat	99 CFU *K. rhizophila*	10 to 40	Successful up to 25×10^6 c/mL
Jurkat	50 CFU *C. albicans*	10 to 40	Successful up to 20×10^6 c/mL
Jurkat	10 CFU *M. orale*	10 to 40	Not successful: PCR inhibition > 15×10^6 c/mL No detection of Mycoplasma spike
Jurkat	10 CFU *M. synoviae*	10 to 35	Not successful: Partial PCR inhibition No detection of Mycoplasma spike
HPBMC	99 CFU *K. rhizophila*	10 to 40	Successful only up to 10×10^6 c/mL
HPBMC	10 CFU *M. arginini*	15.0	Successful
HPBMC	10 CFU *M. orale*	19.1	Successful
HPBMC	99 CFU *P. aeruginosa*	20 and 25	Successful

Table 1: Results of Real-Time PCR detection of respective microbial spikes in varying cell types with respective cell densities.

Tolerance of eukaryotic cell background

Robustness & Ruggedness

HPBMC cells



Successful detection of **99 CFU *P. aeruginosa*** in up to **25 million HPBMC** cells.

Medium: Lympho One T-Cell Expansion

HPBMC: human peripheral blood mononuclear cell (e.g. lymphocytes)

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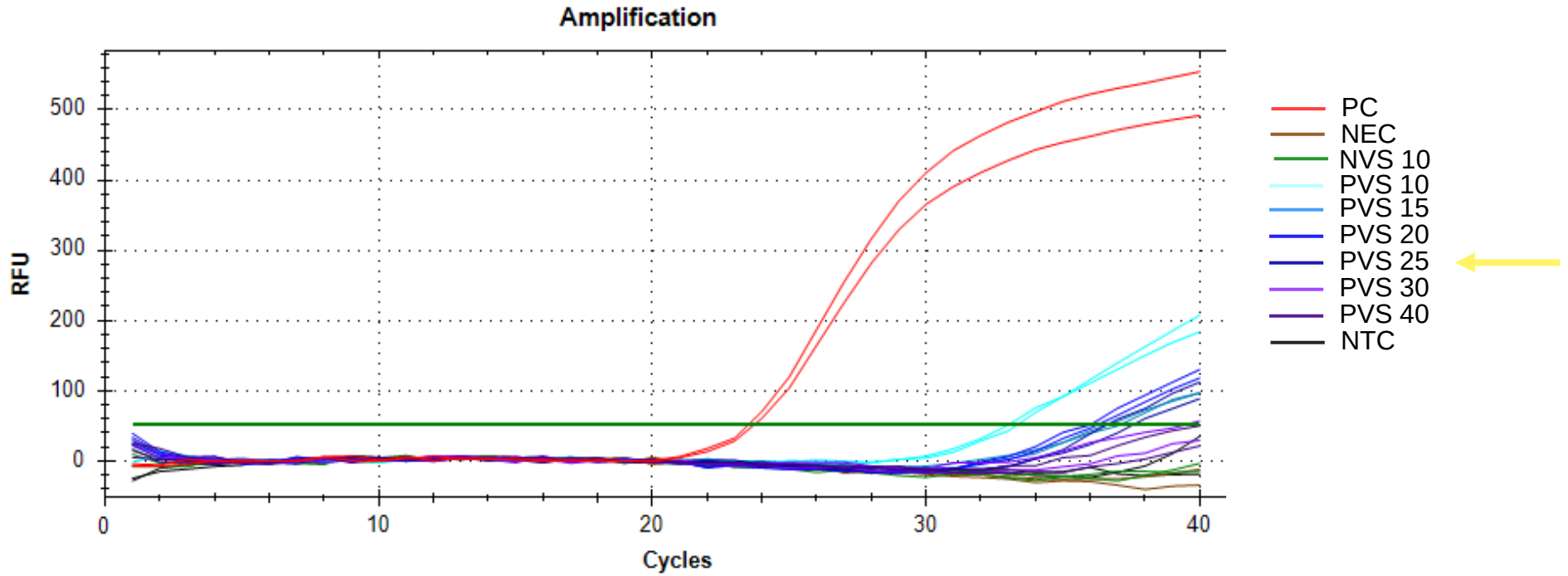
Tolerance of eukaryotic cell background

Robustness & Ruggedness

Jurkat cells



Succesful detection of **99 CFU *Kocuria rhizophila*** in up to **25 million Jurkat** cells.

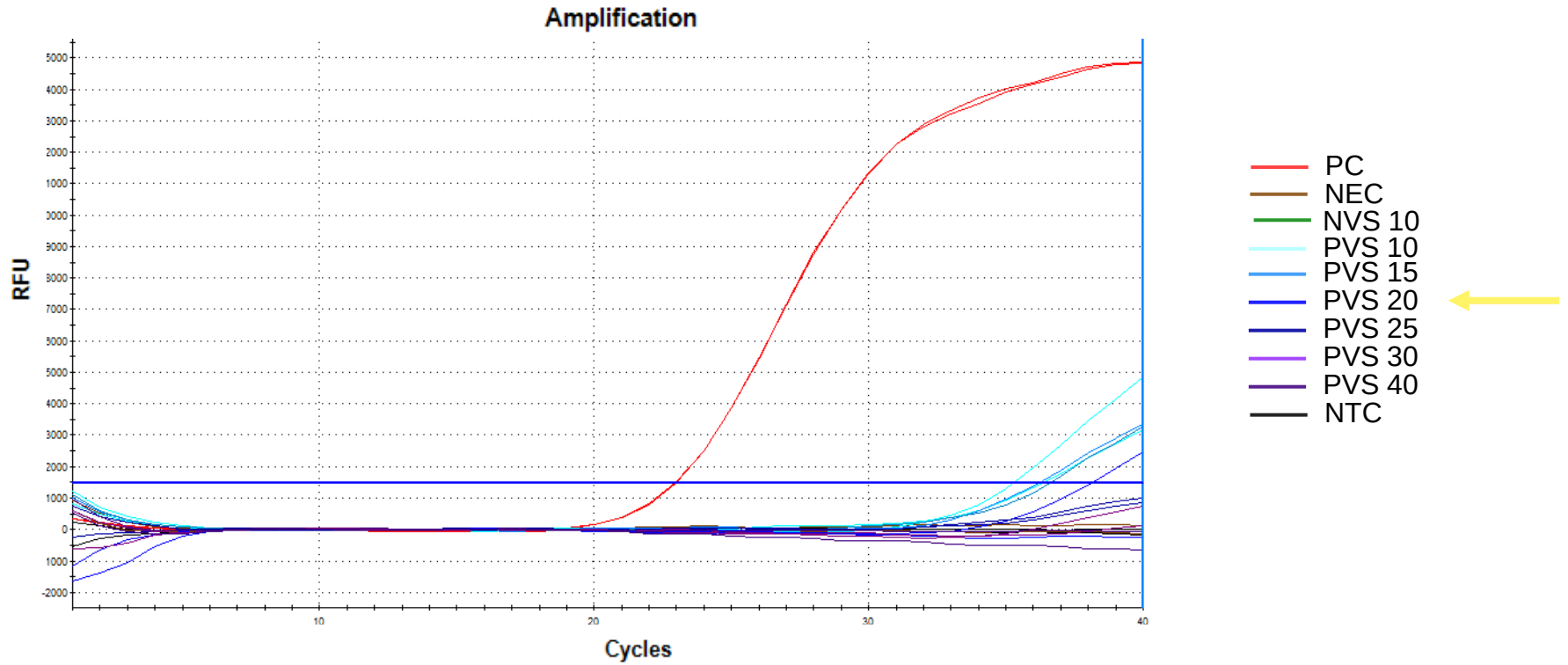
Medium: RPMI 1640

Jurkat: an immortalized human T lymphocyte cell line

Tolerance of eukaryotic cell background

Robustness & Ruggedness

Jurkat cells



Successful detection of **50 CFU *C. albicans*** in up to **20 million Jurkat** cells.

Medium: RPMI 1640

Jurkat: an immortalized human T lymphocyte cell line

Equivalence with compendial culture method

Equivalence

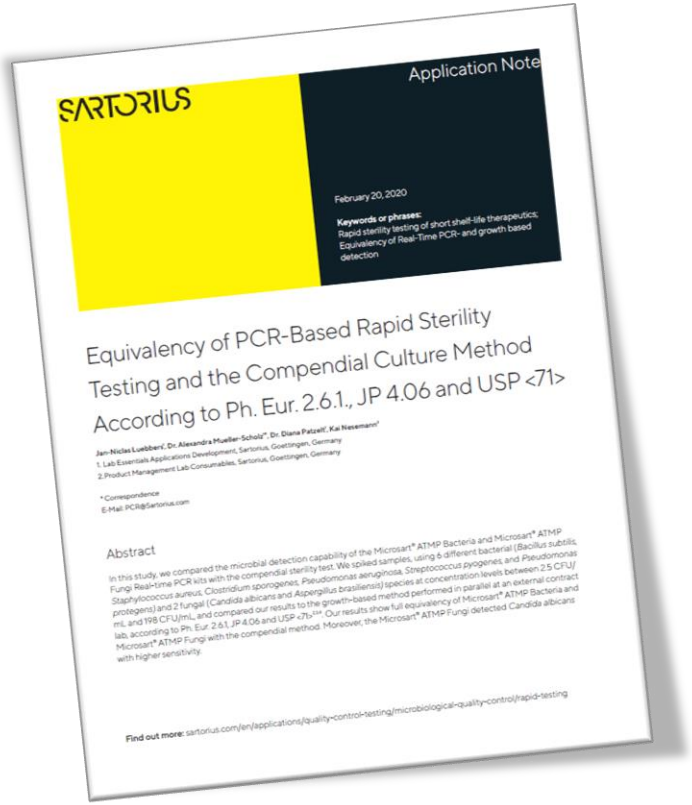
Sartorius

real-time PCR-based detection

?
=

Labor LS

classical sterility test according to EP 2.6.1. and USP<71>



$2x\ LOD_{95}$ LOD_{95} $½\ LOD_{95}$

<u>all 6 mandatory species</u>

- *Bacillus subtilis*
- *Clostridium sporogenes*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Candida albicans*
- *Aspergillus brasiliensis*

<u>recommended extension</u>

- *Streptococcus pyogenes*
- *Pseudomonas protegens*

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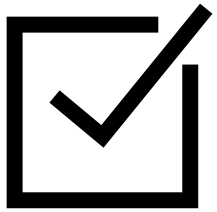
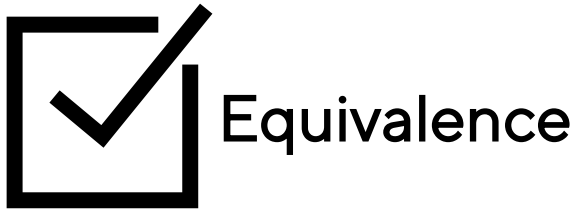
Equivalence with compendial culture method



Equivalence

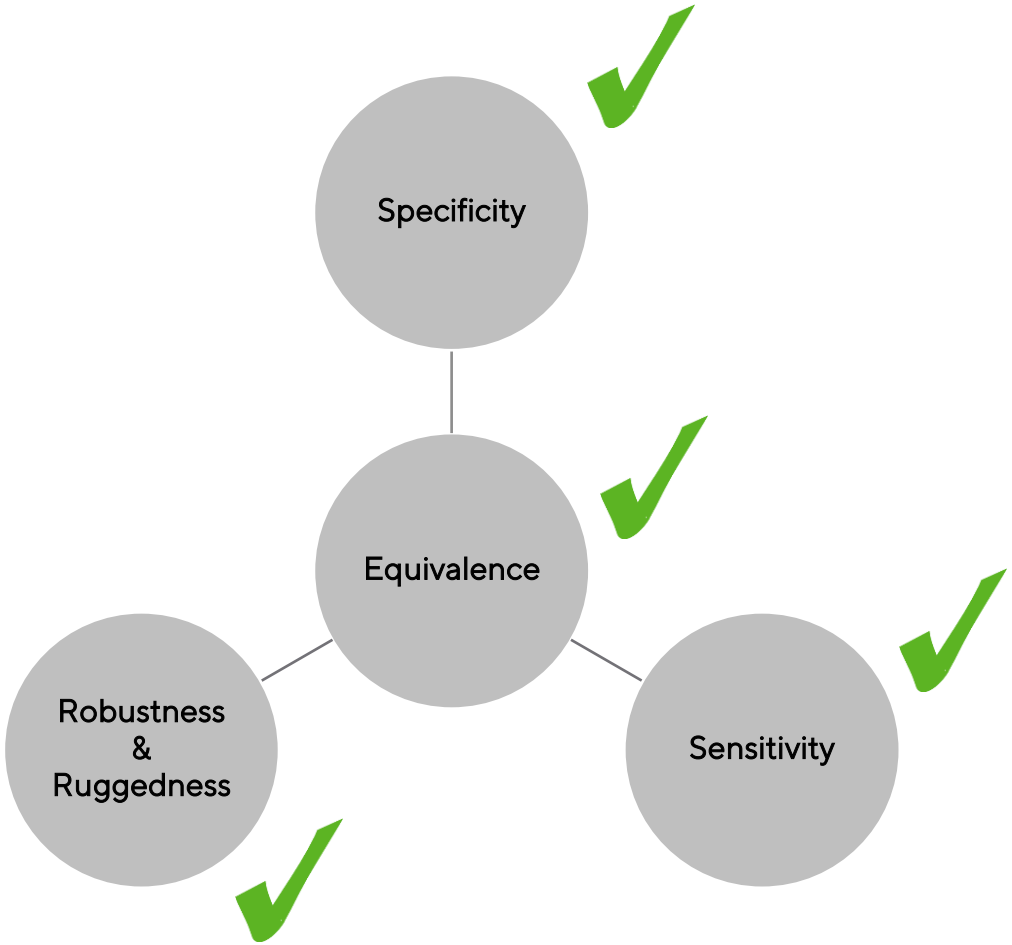
	Microsart® ATMP Bacteria			Compendial culture method (External)		
	2x LOD_{95}	LOD_{95}	$LOD_{95}/2$	2x LOD_{95}	LOD_{95}	$LOD_{95}/2$
Bacillus subtilis	33.16 33.23	34.23 34.32	35.47 34.38	*B. subtilis*	*B. subtilis*	*B. subtilis*
Staphylococcus aureus	35.42 34.13	35.77 35.67	36.56 39.90	*S. aureus*	*S. aureus*	*S. aureus*
Clostridium sporogenes	34.20 34.10	34.87 33.43	35.45 35.61	*C. sporogenes*	*C. sporogenes*	*C. sporogenes*
Pseudomonas aeruginosa	36.40 36.22	36.74 37.96	37.22 No Cq	*P. aeruginosa*	*P. aeruginosa*	Negative
Streptococcus pyogenes	34.89 35.09	35.53 35.93	36.55 35.88	*S. pyogenes*	*S. pyogenes*	*S. pyogenes*
Pseudomonas protegens	34.14 33.28	34.38 34.51	36.52 35.61	Gram – Oxidase +	Gram – Oxidase +	Gram – Oxidase +

Equivalence with compendial culture method



	Microsart® ATMP Fungi			Compendial culture method (External)		
	2x LOD_{95}	LOD_{95}	$LOD_{95}/2$	2x LOD_{95}	LOD_{95}	$LOD_{95}/2$
Candida albicans	32.25	32.27	32.96	*C. albicans*	*C. albicans*	Negative
	31.94	32.12	33.96			
Aspergillus brasiliensis	34.38	37.06	34.94	*A. brasiliensis*	*A. brasiliensis*	*A. brasiliensis*
	32.40	33.17	34.20			

Validation overview



- Sensitivity
  - LOD_{95} - limit of detection
- Specificity
  - Sequence alingment
  - Sample matrix effects/cross reactivity
  - Specificity of PCR with genomic DNA
  - Comparison with compendial method
- Robustness
  - Spiked cell culture samples
  - Device compatibility
  - Detection of free-DNA
  - False positive rate

Simplifying Progress

Coming soon!

Customer validation data
Service Lab **Labor LS**

Labor LS

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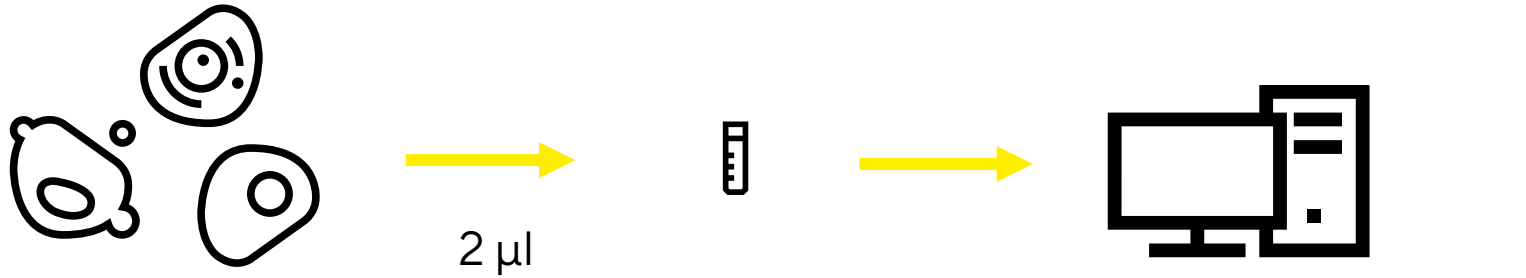
RESEARCH

In-process contamination control of bacteria & fungi

- Key advantages
  - Very robust towards inhibitors
  - No prior DNA extraction required
  - Internal control DNA included in real-time PCR master mix
  - One step preparation

→Quick 'n' Dirty for process monitoring



- Taq-Man® System → reduce false-positive signals
- Duplex assay → reduce false-negative signals
- Universal assay for different real-time PCR cycler → FAM™ and ROX™
- High stability & no freezing → Lyophilized reagents

In-process contamination control of bacteria & fungi

Microsart® RESEARCH Bacteria

Microsart® RESEARCH Fungi

Thank you.

PCR@Sartorius.com

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